DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist

STUDY TYPE:	Acute Injection Toxicity - Mice (OPPTS RED Section V(A)(1)(a))
MRID NO:	449910-02
TEST MATERIAL:	*Bacillus thuringiensis* subspecies *israelensis* Strain EG2215
PROJECT NO:	4721-98
SPONSOR:	Ecogen, Inc., Langhorne, PA 19047
TESTING FACILITY:	STILLMEADOW, Inc., Sugar Land, TX 77478
TITLE OF REPORT:	Acute Intraperitoneal Toxicity Study in Mice
AUTHOR(S):	Janice O. Kuhn, Ph.D., D.A.B.T.
STUDY COMPLETED:	April 7, 1999
GOOD LABORATORY PRACTICE:	GLP Complaint
CONCLUSION:	*Bacillus thuringiensis* subspecies *israelensis* Strain EG2215 appear to be toxic in mice, when dosed at 2.63 x 10^8 cfu/animal, $LD_{50} > 10^6$ spores.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: Batch number DBI-8251-120 contained the microbial pest control agent (MPCA), *Bacillus thuringiensis* subspecies *israelensis* Strain EG2215, with a calculated concentration of 2.63 x 10^{10} cfu/mL[1].

Test Animals: Twenty-five male and female mice strain HSD:CD, were received from Harlan Sprague Dawley, Inc., Houston, TX. They were assigned and weighed, 21.0-29.7 g, and 19.2-24.7 g, respectively at the commencement of the study. The test animals' ages were 19 d. The test animals were housed 5/cage (male and female roomed separately), in polycarbonate with stainless steel wire cover. The test

[1]Calculated and responsibility of the Sponsor.

animal's nutrient source was ™Formulab #5008 (PMI Feeds, Inc.), and water source was municipal tap water supply, analyzed by TNRCC Water Utilities Division.

Methods: Rats were ear-tagged and assigned to treatment groups (Table 1). A stock microbial test substance was prepared (2.63 x 10^9 cfu/g), and diluted for appropriate dose levels (10^6 cfu/0.5 mL, 10^7 cfu/0.5 mL, 10^8 cfu/0.5 mL) per test groups. A second sample of 10^6cfu/0.5 mL was autoclaved (20 min, 115°C). The rats were quarantined (5 d) prior to dosing. The environmental conditions were maintained in the following format: Temperature - 22°C±3°C; Humidity – 30-80%; Light/Dark cycle – 12 h; Air changes - 10-12/h. Treated test animals were weighed on treatment, 7 and final (day 14 or day of death). The test animals who died before scheduled were subjected to gross necropsy. Signs of Pharmacologic/Toxicologic effects and mortality were observed 3 times on treatment day and once daily, up to 14 d. Each surviving treated test animals were euthanized by CO_2 inhalation on day 14.

I. RESULTS

Mortality: Two male and 3 female treated (10^8 cfu) test animals, within 24 h period of inoculation.

Body Weights:

Overall, both male and female test animals gained weight for the duration of the study (Table 1).

Table 1: Average Weight Gains with Time: Dose Level/Animal

Dose Level (CFU)	Male #	Weight (g)			Dose Level (CFU)	Female #	Weight (g)		
		0 d	7 d	14 d			0 d	7 d	14 d
Inactivated 10^8	11 - 15	24.8	31.1	33.6	Inactivated 10^8	16 - 20	22.5	25.4	27.3
10^6	21 - 25	24.3	31.7	34.2	10^6	26 - 30	22.5	25.6	27.8
10^7	31 - 35	22.8	27.9	29.4	10^7	36 - 40	22.4	26.1	27.0
10^8	41 - 45	26.0*	29.7	32.9	10^8	46 - 50	22.4	25.9**	26.8

* N = 3, ** N = 2

Clinical Observation.:

At 4 h and 1 d observation, 5 treated (10^8 CFU) male mice showed a decrease in activity, and 2 deaths occurred respectively. One treated (10^8 CFU) female mice displayed ptosis, diarrhea and soft feces, and 5 displayed a decreased in defecation/activity at 4 h observations. On day 1, 2 female mice displayed a decreased in defecation and 3 mortalities.

Gross Necropsy:

Necropsy studies in the following treated/terminated test animals were

- Male 42 — Liver pale and dark at edges; spleen dark at distal tip
- Male 44 — Liver pale and dark at edges; spleen dark at distal tip; red-brown gel in intestinal tract
- Female 46 — Stomach mucosa reddened; spleen dark at distal tip; liver pale and dark at edges; red-brown gel in intestinal tract
- Female 48 — Liver pale and dark at edges; spleen dark at distal tip; red-brown gel in intestinal tract
- Female 49 — Lungs mottled; liver pale and dark at edges; large intestine empty

III. DISCUSSION

Within 24 h of treatment, 5 (2 males and 3 females) mortalities occurred, when the treated test animals were dosed at 10^8CFU. Necropsy studies on the decease animals showed a pale liver with darkness around the edges, darken distal tip on spleen, reddish-brownish coloring in the intestinal tract, reddish mucosal stomach, mottled lungs, and emptied large intestines. *Bacillus thuringiensis* subspecies *israelensis* Strain EG2215 appears to be toxic and/or pathogenic in mice, when dosed at 10^8 cfu/animal, $LD_{50} > 10^7$ spores. The packet classification is ACCEPTABLE.

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Chemical: Bacillus thuringiensis subspecies israelensis strain EG2215

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